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**Relationships of Growth and Age to Organ Weights in Rats**

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THE success of an animal depends on constant physiological and behavioral responses to the environment. In the case of mammals, responses to other members of the same species are of great importance. Selye (1950) proposed that abnormalities in the process of adaptation may induce physiological changes. This proposal has led to many studies (reviewed by Christian, 1963; Christian, Lloyd and Davis, 1965; and Horn, 1967) on possible regulation of mammalian populations by means of endocrine feedback mechanisms. These studies usually have hypothesized that when environmental conditions fail to limit population growth, generalized systemic effects of chronic non-specific stress contribute to population stabilization. This stabilization results from decreased natality and increased mortality.

The purpose of our study was to 1) compare the behavioral responses of animals in populations of low and high density, 2) determine the effects of otic, optic, and olfactory interaction between populations of high and low density, 3) determine the effective mechanism of population stabilization in this study, 4) determine whether organ weights responded consistently to population densities, and 5) interpret differences in organ weights with respect to growth rate and observed behavioral characteristics.

METHODS AND MATERIALS

*Colony.* Black-hooded rats (Long-Evans strain) were used to establish three populations: 1) Dense (D), 2) Non-dense (ND), a low-density population adjacent to the dense population, and 3) control (C), a low-density population not exposed to sensory interaction with the D populations.

Five replicas of each condition were established with randomly assigned litter mates of approximately the same age. D cages were initiated with 12, 14, or 16 weanlings; at no time were dense  $F_1$  ( $DF_1$ ) animals removed from the experiment. ND and C cages were initiated with one half the number of animals placed in the companion D cage;  $F_1$  animals in ND and C cages were removed from the experiment at approximately 21 days of age. The number of males and females in each population is given in Table 1.

TABLE 1  
Mortality

Population	Males		Females		Total	
	No.	Deaths	No.	Deaths	No.	Deaths
Dense	33	3(9%)	35	9(25.7%)	68	12(17.7%)
Non-dense	16	1(6.3%)	17	2(11.8%)	33	3(9%)
Control	16	1(6.3%)	17	4(23.5%)	33	5(16%)
Dense, $F_1$ generation	42	6(14.3%)	30	3(10%)	72	9(12.5%)
Total	107	11(10.3%)	99	18(18.2%)	206	29(14.1%)

Data for  $F_1$  animals include those that were killed after being marked and admitted to the experiment, as well as those that died of natural causes. Cannibalism prohibited differentiation in the dense cages. Deaths in the parent generation at all densities are due to natural causes.

The 15 experimental cages were constructed of wire mesh on aluminum frames  $36 \times 12 \times 10$  in. and placed on vertical racks of five cages each. Cage interiors were not subdivided. Unlimited food, water, and nesting material were provided. The water source was always at the same end of the cage; food and nesting materials were scattered on the cage floor.

The colony was maintained for approximately six months.

*Sensory interaction.* Racks with D and ND populations were placed back-to-back so that companion cages were separated only by wire-mesh boundaries. This arrangement permitted all modes of sensory interaction except touch. Control cages were maintained in a separate room in order to prohibit sensory interaction between experimental and control animals.

*Environment.* Photoperiods were regulated by timing devices in each room. Temperature and relative humidity remained between 70 and 75 F. and 50 to 55 per cent, respectively.

*Behavior.* Individuals, marked by clipped toes and shaved pelage, were observed through one-way glass for periods that varied from  $\frac{1}{2}$  to 4 hrs. Descriptions of agonistic interactions, as well as general behavior, were recorded.

*Growth rate.* All individuals were weighed every 2 weeks. The number of days required for an increase of body weight from 100 to 180 g was calculated for each individual. This weight interval was chosen because it included the largest number of both parent and DF<sub>1</sub> animals.

*Organ weights.* All rats were killed at the termination of the experiment, autopsied immediately, and freshly excised organs (pituitary, thymus, spleen, thyroid and paired adrenals, kidneys, submaxillary glands, testes and seminal vesicles) were weighed to the nearest 0.1 mg.

Statistical analyses are based on 176 sexually mature individuals, 114 parents and 62 DF<sub>1</sub> animals. Sexual maturity was determined for males by position and size of testes, size of seminal vesicles and cytological evidence of spermatogenesis; and for females by size and condition of uterus, presence of corpora lutea, placental scars or embryos. Only DF<sub>1</sub> animals aged 80 days or older were used in the statistical analysis.

## RESULTS

*Behavior.* Behavior patterns in the D populations closely paralleled those described by Calhoun (1961), except that we found no evidence of withdrawal into homosexuality. Males that exhibited homosexual activity also exhibited heterosexual activity. As the population increased, infanticide by both males and females increased. The most obvious change was a breakdown in maternal behavior. Nest building declined and finally ceased. Mothers displayed disoriented retrieving patterns by randomly depositing young throughout the cage. These young soon died or were eaten. The upper asymptote of population density differed slightly in each of the five D cages. When the density in any D cage reached this level, however, no infants in the D cages survived more than a few hours after birth.

General levels of activity were lower in D than in ND or C cages. Rats in D cages were usually grouped in large aggregates, or huddles, similar to those described by Lloyd and Christian (1967). Individuals did not engage in periods of sustained movement but spent most of the time either sleeping or clinging to the sides of the cages. This lethargy was interrupted at apparently random intervals by bursts of sexual activity that quickly involved almost every animal in the cage. This activity appeared to be socially motivated, as described by Grant (1963). He identified behavior sequences common to both male-female and male-male sexual behavior and suggested that male sexual motivation is aroused in social situations. Indiscriminant mounting by males of both males and unwilling females provoked brief scuffles and squeaks, but little or no wounding. The absence of intense fighting is characteristic of the laboratory rat. (Barnett, 1963).

Feeding patterns were frequently disrupted in D cages. When rats were molested or jostled during feeding, no matter whether the physical contact was accidental or resulted from directed social encounters, feeding animals immediately dropped the food pellet and began intensive face washing. Following a few abortive attempts to feed, the animals ceased eating and retreated to the huddle, either climbing to the top or burrowing underneath.

*Mortality, reproduction, and infant survival.* Mortality by sex, population condition, and generation is shown in Table 1. No adult animals died as a result of fighting or wounding. More adult females than males died in all populations. In the DF<sub>1</sub> generation more males died. Deaths in the DF<sub>1</sub> generation are given only for those individuals that survived more than 21 days and had been assigned a number.

There was no evidence of significantly decreased fertility in D populations. Because of cannibalism, it was impossible to accurately record the number of young per litter or the total number of litters born in D cages. Direct observation showed that infants were frequently destroyed as soon as they were born. The only valid information about comparative natality was obtained at autopsy. The mean number of embryos per female was smaller in D than in either ND or C cages; but the differences were not significant (Table 2). The number of females that were pregnant or had recent placental scars did not indicate a significant reduction in fer-



tility associated with population density.  $DF_1$  females began to reproduce at the same age as did their parents (approximately 90 days) and corpora lutea were present in the ovaries of all  $DF_1$  fe-

TABLE 2  
Mean number of embryos

	D (14)	ND (6)	C (8)	$DF_1$ (10)
Mean $\pm$ S.E.	6.8 $\pm$ 1.3	9.7 $\pm$ 1.8	9.4 $\pm$ 0.8	8.4 $\pm$ 1.2

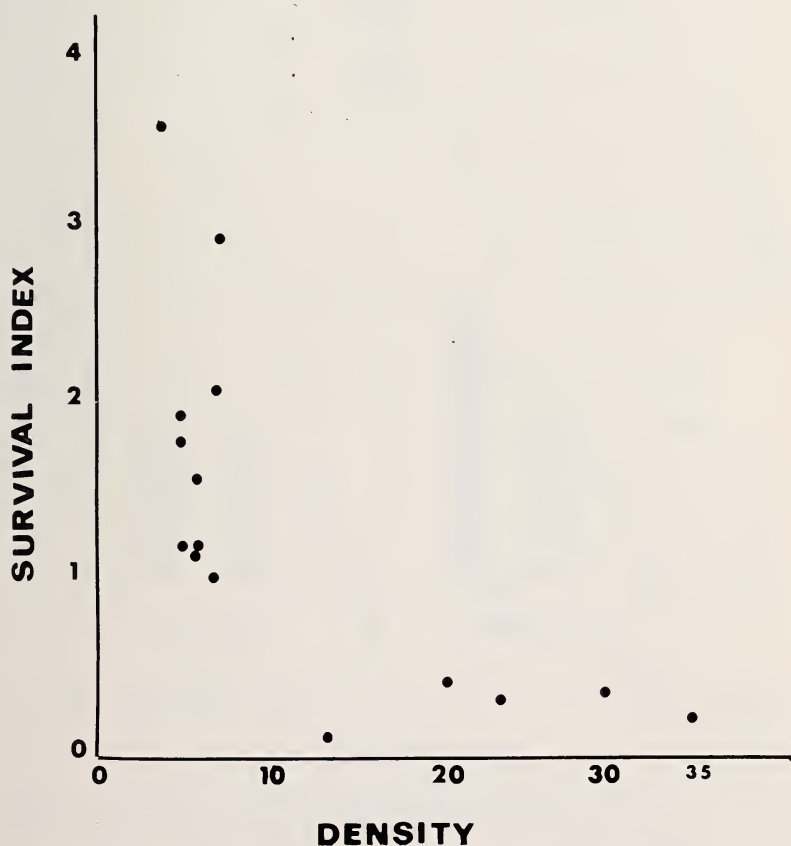


Fig. 1. Infant survival index plotted against final number of rats per cage. See text for method of calculation of index.

males at autopsy. Cytological examination showed abundant evidence of spermatogenesis in all  $DF_1$  males. We found no evidence of delayed sexual maturation in either sex that could be associated with population density.

An infant survival index was calculated for each cage. The total number of weaned individuals was divided by the total number of possible 21-day gestation periods for each female of reproductive age. The survival index, plotted against the final number of ani-

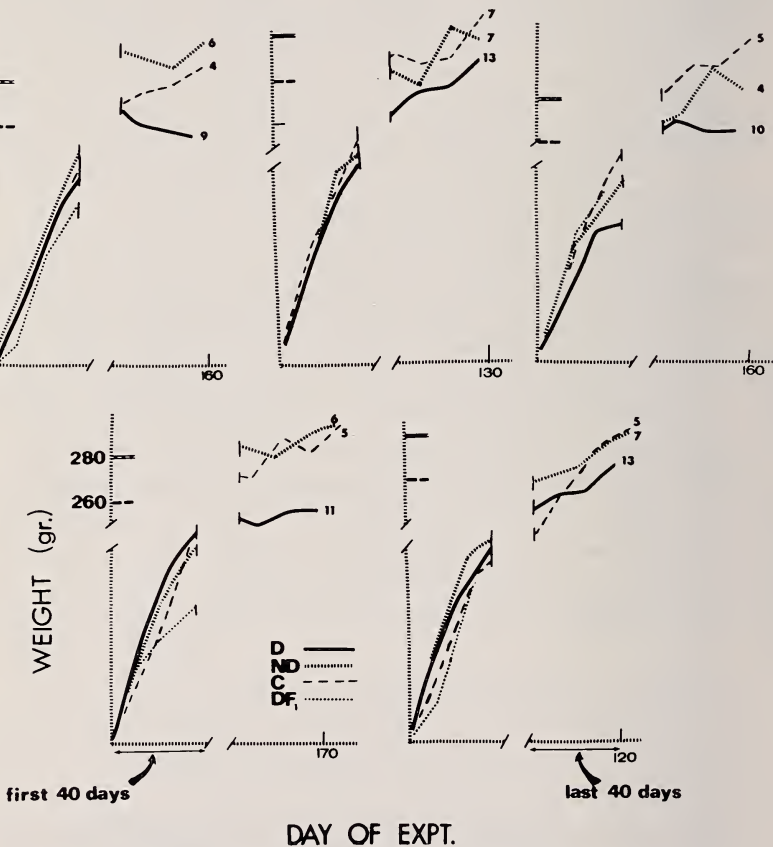


Fig. 2. Weight averages for each of five replicas of D, ND, and C populations.  $DF_1$  rats are shown separately from their parents. All lines begin at an initial weight of 100 g. The first half of each line represents the first forty days after reaching a mean weight of 100 g; the last half of each line represents the last 40 days of the experiment. In this figure and all following figures D refers to dense parents.

imals in each cage shows that survival to weaning age was greatly reduced in D cages, and proportionately less of the reproductive potential was realized (Fig. 1). The points on this figure represent a discontinuous function and cannot be connected by a line with any validity. When density was low, the survival index was one or greater; when density was high, it was less than 0.5. Since this difference cannot be explained by a significantly decreased birth rate, infant mortality was apparently increased. When  $DF_1$  animals did

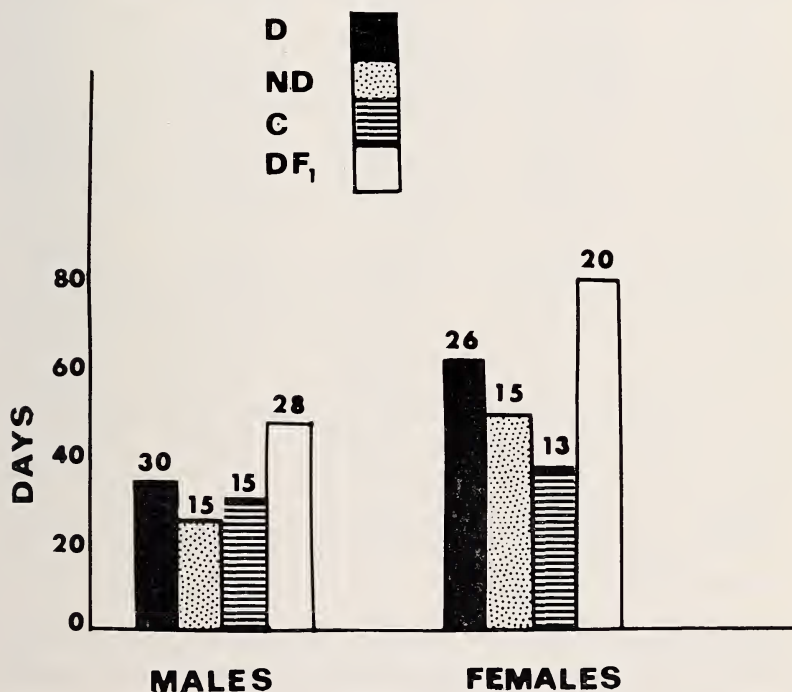


Fig. 3. The mean number of days required to gain weight from 100 to 180 g. The number of animals is given.

survive to weaning age, their mortality rate was lower than the parent generation in both D and C cages (Table 1).

*Growth rate.* Animals in D cages demonstrated a slower growth rate than those in either ND or C cages.

Averages of biweekly weights were consistently lower for D populations; no consistent trend appeared between ND and C populations (Fig. 2). Mean weights were calculated separately for D parents (DP) and DF<sub>1</sub> generations. Mean death weights are given in Table 3.

The slower rate of growth of D animals is further reflected in the mean number of days required to gain in weight from 100 to 180 g. D animals required a greater number of days than did either ND or C animals (Fig. 3). The most striking decrease in rate of

TABLE 3  
Absolute organ weights of males (mean±S.E.)

		Dense (N=30)	Non-dense (N=15)	Control (N=15)	Dense, F <sub>1</sub> generation (N=37)
Weight	g	281.1	308.9	295.7	198.9
Adrenals	mg	37.7±1.6	37.0±1.9	41.9±1.4	32.5±1.1
Pituitary	mg	8.8±0.3	8.8±0.4	10.2±0.7	7.5±0.3
Thyroid	mg	11.8±0.8*	12.6±1.5	15.4±1.1*	10.7±0.7
Spleen	g	0.7±0.04	0.7±0.42	0.6±0.05	0.7±0.03
Thymus	g	0.1±0.01*	0.1±0.14	0.2±0.02*	0.2±0.01
Kidneys	g	2.5±0.08	2.8±0.11	2.7±0.02	1.9±0.05
Submax.	g	0.6±0.02	0.6±0.02	0.6±0.03	0.5±0.02
Testes	g	2.6±0.06	2.8±0.05	2.7±0.07	2.2±0.04
Seminal Vesicles	g	1.0±0.06	1.2±0.10	1.1±0.06	0.6±0.05

\*significant difference ( $P<.05$ )

growth appears in the DF<sub>1</sub> animals. This fact suggests that animals born into a dense population evidence a slower rate of growth than animals assembled into a dense population.

*Organ weights.* Organ weights were considered both as absolute and as relative values (mg organ wt. per g body weight). Mean values were compared for each population by means of the Student "t" test. Whereas significant differences did appear between the averages of some organ weights, they appear to be the result of randomness and not of any specific physiological syndrome (Tables 3-8).



TABLE 4  
Relative organ weights of males (mean $\pm$ S.E.)

		Dense (N=30)	Non-dense (N=15)	Control (N=15)	Dense, F <sub>1</sub> generation (N=37)
Weight	g	281.1	308.9	295.7	198.9
Adrenals	mg	0.14 $\pm$ 0.01	0.12 $\pm$ 0.01*	0.14 $\pm$ 0.00*	0.17 $\pm$ 0.00
Pituitary	mg	0.03 $\pm$ 0.01	0.03 $\pm$ 0.00	0.35 $\pm$ 0.00	0.38 $\pm$ 0.00
Thyroid	mg	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00	0.05 $\pm$ 0.00	0.05 $\pm$ 0.00
Spleen	g	2.53 $\pm$ 0.13	2.32 $\pm$ 0.10	2.17 $\pm$ 0.16	3.64 $\pm$ 0.16
Thymus	g	0.52 $\pm$ 0.03	0.48 $\pm$ 0.05	0.63 $\pm$ 0.06	1.00 $\pm$ 0.74
Kidneys	g	8.92 $\pm$ 0.02	9.12 $\pm$ 0.20	8.94 $\pm$ 0.19	9.75 $\pm$ 0.24
Submax.	g	2.15 $\pm$ 0.62*	1.88 $\pm$ 0.06*	2.01 $\pm$ 0.12	2.58 $\pm$ 0.12
Testes	g	9.28 $\pm$ 0.24	9.03 $\pm$ 0.26	9.35 $\pm$ 0.35	11.5 $\pm$ 0.30
Seminal Vesicles	g	3.62 $\pm$ 0.18	3.91 $\pm$ 0.34	3.65 $\pm$ 0.21	3.11 $\pm$ 0.19

\*significant difference ( $P<.05$ )TABLE 5  
Absolute organ weights of nonpregnant females (mean $\pm$ S.E.)

		Dense (N=12)	Non-dense (N=8)	Control (N=4)	Dense, F <sub>1</sub> generation (N=15)
Weight	g	196.8	219.2	230.0	155.0
Adrenals	mg	*54.2 $\pm$ 2.0†	63.8 $\pm$ 4.0	65.8 $\pm$ 3.5†	*43.5 $\pm$ 2.6
Pituitary	mg	9.9 $\pm$ 0.90	10.2 $\pm$ 0.70	12.1 $\pm$ 2.9	8.4 $\pm$ 0.8
Thyroid	mg	10.0 $\pm$ 1.00	8.1 $\pm$ 0.80†	12.2 $\pm$ 1.3†	9.7 $\pm$ 0.8
Spleen	g	0.8 $\pm$ 0.09	0.6 $\pm$ 0.05	0.7 $\pm$ 0.08	0.6 $\pm$ 0.06
Thymus	g	0.1 $\pm$ 0.01	0.1 $\pm$ 0.06	0.2 $\pm$ 0.09	0.2 $\pm$ 0.02
Kidneys	g	*2.0 $\pm$ 0.07	2.1 $\pm$ 0.12	2.4 $\pm$ 0.28	*1.5 $\pm$ 0.07
Submax.	g	*0.5 $\pm$ 0.04	0.4 $\pm$ 0.03	0.4 $\pm$ 0.04	*0.4 $\pm$ 0.02

\*†significant difference ( $P<.05$ )

TABLE 6  
Absolute organ weights of pregnant females (mean $\pm$ S.E.)

		Dense (N=14)	Non-dense (N=7)	Control (N=9)	Dense, F <sub>1</sub> generation (N=10)
Weight	g	210.7	236.4	235.3	181.9
Adrenals	mg	53.6 $\pm$ 4.4	63.4 $\pm$ 3.6	62.0 $\pm$ 5.3	47.4 $\pm$ 3.3
Pituitary	mg	9.1 $\pm$ 0.6†	10.3 $\pm$ 0.8	12.6 $\pm$ 1.0†	8.0 $\pm$ 0.8
Thyroid	mg	10.3 $\pm$ 0.9	9.2 $\pm$ 0.65†	11.7 $\pm$ 0.8†	9.6 $\pm$ 0.83
Spleen	g	0.7 $\pm$ 0.04	0.7 $\pm$ 0.05	0.8 $\pm$ 0.11	0.6 $\pm$ 0.06
Thymus	g	0.1 $\pm$ 0.06	0.1 $\pm$ 0.02	0.1 $\pm$ 0.03	0.2 $\pm$ 0.03
Kidneys	g	*1.9 $\pm$ 0.08†	*2.2 $\pm$ 0.09	2.4 $\pm$ 0.11†	1.7 $\pm$ 0.95
Submax.	g	0.5 $\pm$ 0.03	0.5 $\pm$ 0.04	0.5 $\pm$ 0.02	0.5 $\pm$ 0.05

\*†significant difference (P<.05)

TABLE 7  
Relative organ weights of nonpregnant females (mean $\pm$ S.E.)

		Dense (N=12)	Non-dense (N=8)	Control (N=4)	Dense, F <sub>1</sub> generation (N=15)
Weight	g	196.8	219.2	230.0	155.0
Adrenals	mg	0.28 $\pm$ 0.016	0.29 $\pm$ 0.020	0.29 $\pm$ 0.017	0.28 $\pm$ 0.014
Pituitary	mg	0.05 $\pm$ 0.004	0.47 $\pm$ 0.003	0.05 $\pm$ 0.010	0.06 $\pm$ 0.005
Thyroid	mg	0.05 $\pm$ 0.004†	*0.04 $\pm$ 0.004†	*0.05 $\pm$ 0.004	0.07 $\pm$ 0.008
Spleen	g	4.24 $\pm$ 0.480†	2.83 $\pm$ 0.200†	3.00 $\pm$ 0.250	3.76 $\pm$ 0.280
Thymus	g	*0.57 $\pm$ 0.075	0.46 $\pm$ 0.031	0.86 $\pm$ 0.369	0.98 $\pm$ 0.112
Kidney	g	10.10 $\pm$ 0.400	9.34 $\pm$ 0.360	10.40 $\pm$ 0.400	10.10 $\pm$ 0.400
Submax.	g	2.45 $\pm$ 0.240	1.96 $\pm$ 0.100	1.90 $\pm$ 0.070	2.30 $\pm$ 0.110

\*†significant difference (P<.05)

TABLE 8  
Relative organ weights of pregnant females (mean $\pm$ S.E.)

		Dense (N=14)	Non-dense (N=7)	Control (N=9)	Dense, F <sub>1</sub> generation (N=10)
Weight	g	210.7	236.4	235.3	181.9
Adrenals	mg	0.26 $\pm$ 0.019	0.28 $\pm$ 0.025	0.26 $\pm$ 0.020	0.27 $\pm$ 0.025
Pituitary	mg	0.04 $\pm$ 0.003	0.05 $\pm$ 0.005	0.05 $\pm$ 0.006	0.04 $\pm$ 0.005
Thyroid	mg	0.05 $\pm$ 0.005	0.04 $\pm$ 0.004	0.05 $\pm$ 0.003	0.05 $\pm$ 0.006
Spleen	g	3.11 $\pm$ 0.170	3.19 $\pm$ 0.210	3.55 $\pm$ 0.430	3.55 $\pm$ 0.330
Thymus	g	0.62 $\pm$ 0.028	0.56 $\pm$ 0.055	0.60 $\pm$ 0.010	0.91 $\pm$ 0.165
Kidneys	g	8.83 $\pm$ 0.330†	9.36 $\pm$ 0.370	10.30 $\pm$ 0.500†	9.29 $\pm$ 0.500
Submax.	g	2.38 $\pm$ 0.120†	2.00 $\pm$ 0.130†	2.04 $\pm$ 0.070	2.72 $\pm$ 0.360

\*†significant difference ( $P < .05$ )

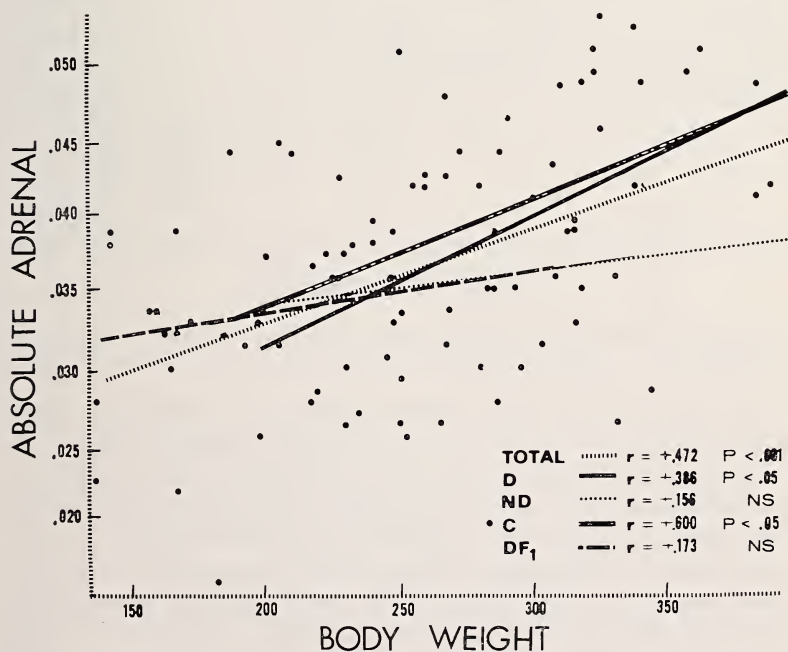


Fig. 4. Correlations of male adrenal weights (g) with body weights. (NSD among the slopes).

The previously established sex differences in pituitary and adrenal weights (Farris and Griffith, 1949) were found. Absolute weights of female pituitary glands were greater than those of males (NSD); because of smaller body size, relative weights of female pituitary glands were significantly greater ( $p < .05$ ) than those of males. Both absolute and relative values of female adrenal weights

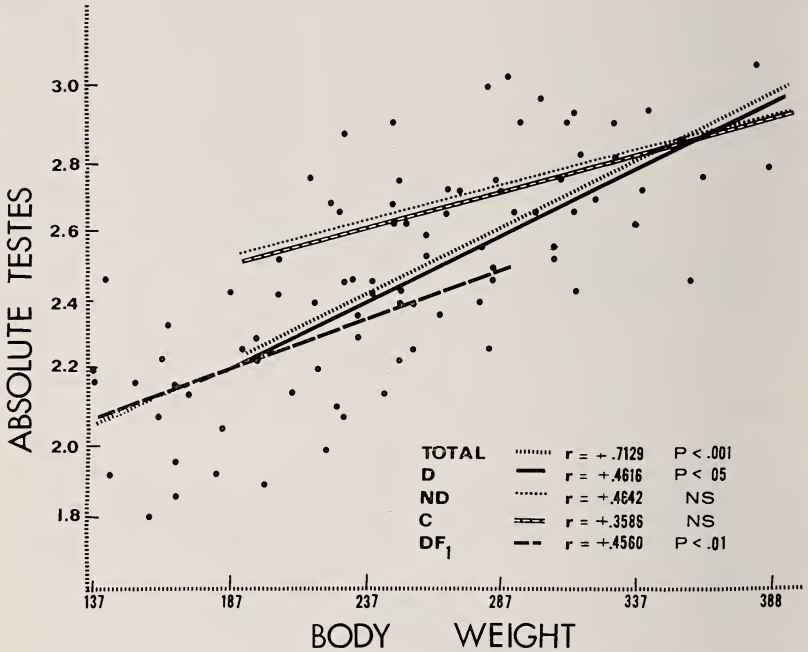


Fig. 5. Correlation of testes weights with body weights. (NSD among the slopes).

are greater ( $p < .05$ ) than those of males. No significant differences in relative or absolute values of adrenal weights resulted between pregnant and non-pregnant females within each population.

Tables 3-8 show the lack of consistent trends with respect to either density or sex in the weights of other organs studied.

In order to determine whether the relationship between selected organs and body weight had been altered by experimental conditions, an analysis of covariance was used. Females evidenced weak organ to body weight relationships (low correlation coefficients), undoubtedly caused by the variability of body weight due



to reproductive condition. With males, no significant difference was found among slopes when absolute weights of adrenals and testes were correlated with body weight (Figs. 4-5). A significant difference did result among slopes when relative weights of adrenals ( $P=.04$ ) and testes ( $p<.001$ ) were correlated with body weight (Figs. 6-7). No significant difference was found among

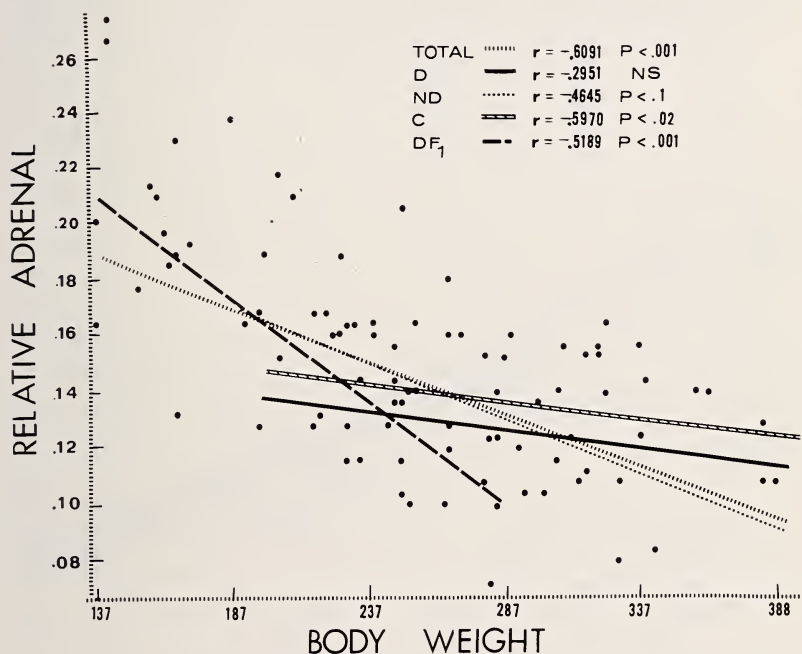


Fig. 6. Correlation of male adrenal weights (mg/g) with body weight. A significant difference ( $P, .04$ ) is found among the slopes.

slopes when relative weights of adrenals were correlated with relative weights of testes (Fig. 8).

The conversion of absolute organ weights to relative values did not compensate equally for differences in sizes of animals, as shown by the negative correlation in Figs. 6-7. With the exception of the pituitary (not shown), this conversion reduced the scatter about the regression line, as indicated by the higher "r" values shown in Figs. 6-7. Pituitary glands appeared to be more constant in size, and therefore conversion of pituitary weights to relative values resulted in decreased "r" values.

*Sensory interaction.* The effect of sensory interaction between a D and an adjacent ND population appeared to be negligible. D populations that exhibited distorted behavior patterns did not induce these patterns in adjacent ND populations. This interaction did not elicit physiological responses that resulted in significantly or consistently disparate mean weights of organs (Tables 3-8).

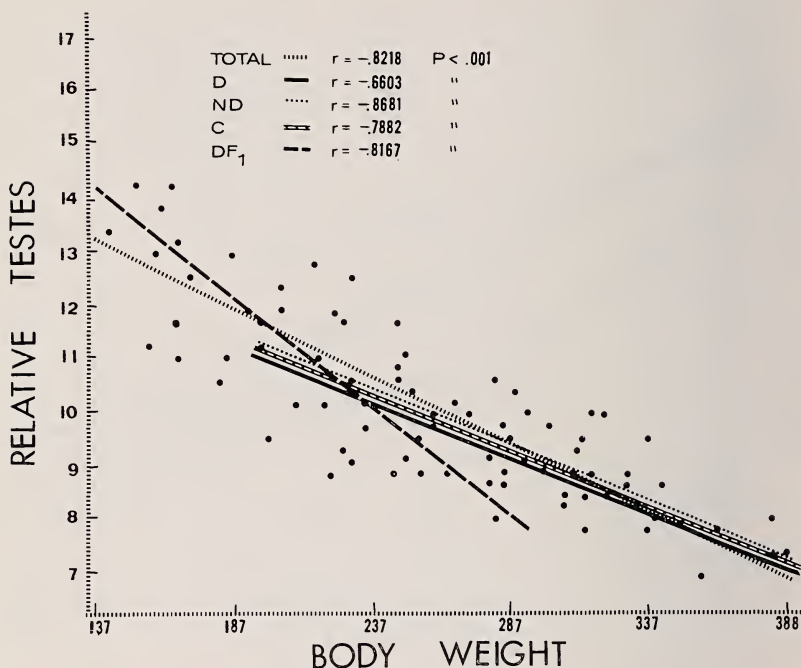


Fig. 7. Correlation of testes weights (mg/g) with body weight. A significant difference ( $P < .001$ ) occurs among the slopes.

#### DISCUSSION

The primary effects of high population density in this study were marked behavioral distortions and reduced growth rate.

*Effect of density on behavior.* Differences in behavior between animals in D, ND, and C populations were largely a matter of degree, both qualitatively and quantitatively. These differences were not reflected in significantly different organ weights. The distorted behavior that was continually observed in D populations was also occasionally observed in ND and C populations. Such behavior

appeared to result from 1) the limitation of activities through spatial restrictions and 2) experimental conditions which produced conflict and disruption of those stimuli necessary to elicit sequences of animal behavior. (Van Vleck and Gentle, unpublished manuscript).

*Population stabilization as a function of behavior.* Population stabilization in this study was a function of behavioral distortions and not of decreased fertility or increased mortality of adults.

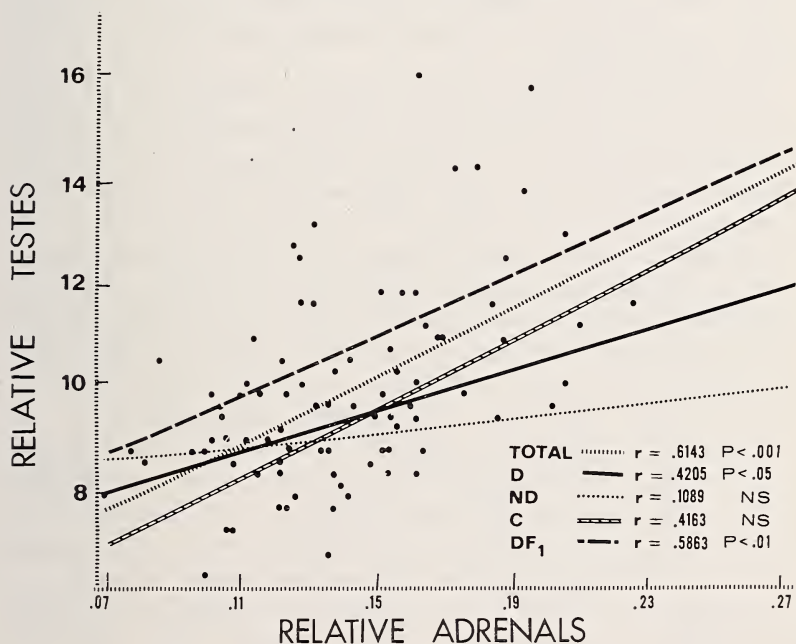


Fig. 8. Correlation of testes weights (mg/g) with adrenal weights (mg/g). NSD among the slopes.

The increase of population terminated when infant survival dropped to zero. The primary factors in increased infant mortality were infanticide and the degeneration of maternal behavior. We found no significant evidence of the depression of natality such as significantly decreased litter size, increased intra-uterine mortality or delayed sexual maturation, as reported by Hoffman (1958) Helmreich (1960), Christian et al. (1965), and Crowcroft (1966). Although fewer embryos per female were found in D populations,

the difference was not significant and not sufficient to cause population stabilization. The reproductive effort was similar at both low and high density. These results agree with Southwick (1955), Christian (1956) and Hoffman (1958), who found that the main factor limiting population size was infant mortality.

Mortality was slightly higher in D cages. However, the difference was not as great as would have been expected if a depression of natural defence mechanisms (Speirs and Meyer, 1949; Vessey, 1964) had occurred. There was no evidence of splenic hypertrophy, a condition which has been associated with changes in viability (Chitty, 1957).

*Relationship of behavior to growth rate.* Behavioral responses also may have contributed to the reduced growth rate of animals in D cages (Figs. 2-3). The frequent interruption of feeding behavior observed in this study suggested that caloric intake might have been reduced. However, fat deposits were present in the mesenteries of all animals in all cages, which indicated that no animal was suffering from serious malnutrition. Crowcroft (1966) reported fat deposits in underfed mice. The slower growth rate may have resulted from reduced somatic growth, reduced fat deposition, or reduced caloric intake. Steinberg and Watson (1960) found reduced growth may often result simply from disturbance and handling of laboratory rats. Calhoun (1962) suggested that growth rate and adult weight in wild Norway rats may be inversely proportional to the degree of social disturbance and instability. He attributed reduced growth to an endocrine-stress response, but stated that he had no proof that caloric intake was not reduced.

Growth rate, as well as fat deposition, are good indices of the general physical condition of an animal. Growth rate naturally decreases as a result of aging, however, inhibited growth rate at any age may result from environmental, endocrine, or behavioral factors. Calhoun's (1962) suggestion that growth rate is related to social stability was based on records of lengths, weights, and ages in a population of Norway rats. He used the ratio of length: weight of an index of both growth rate and maturity. He was able to determine differential growth rates by plotting length: weight ratios (LWR) against known age. The curve resembled an inverse image of either length or weight plotted against age: i.e., the thinner the animal, the higher the LWR, or the heavier the animal the lower the LWR. By using continuous records, he found that younger



animals were proportionally thinner than older animals. More important, he found that animals that grew slowly were thinner at any age level than animals that grew more rapidly. This was true even though the slower growing animals had fat deposits in the mesenteries. These facts indicate that unless organ weights are considered within the context of growth rate and age structure of a population, spurious interpretations may arise.

*Relationship of growth rate and age structure to the interpretation of differences of organ weights.* Statistical evaluation of organ weights in this study produced evidence to support logical but incompatible interpretations. For instance, when considering only mean relative weights of male adrenals (Tables 3-8), the larger value for  $DF_1$  males suggests hypertrophy of adrenals of young rats born into a dense population. Considering mean relative weights of testes, however, it appears that "hypertrophy" of testes also occurred in the  $DF_1$  generation. Simultaneous hypertrophy of adrenals and testes is contrary to any proposed mechanism of intrinsic population control. Theoretically weights of these two organs should respond in opposite fashion to population density (Christian et al., 1965).

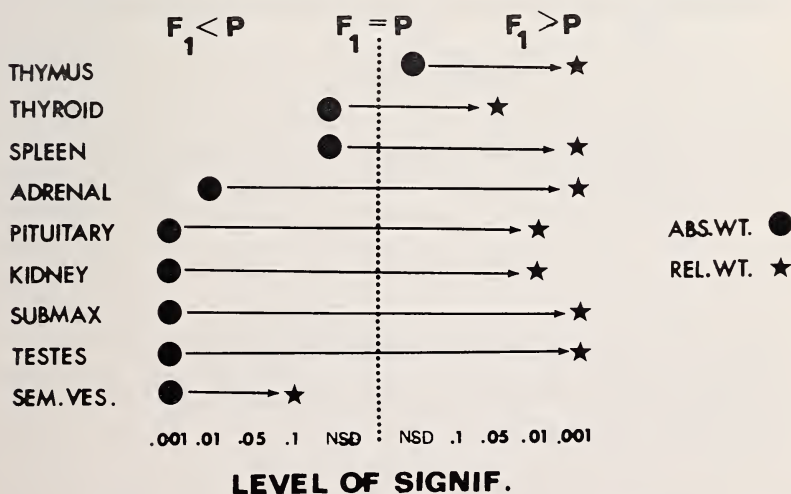


Fig. 9. Comparison of organ weights of DP and  $DF_1$  males. This shows the statistical magnitude of difference between the smaller absolute weights and larger relative weights of organs of  $DF_1$  rats. Actual mean values are shown in Tables 3-6.

The phenomenon of apparent hypertrophy was consistent in other male organs. We found that mean absolute organ weights were smaller in young adults ( $DF_1$ ) than in older adults (DP), but that mean relative organ weights were greater. Figure 9 shows the statistical magnitudes of difference between generations. Two exceptions to this generalization were absolute weights of thymus and relative weights of seminal vesicles. Both exceptions are predictable as a function of the ontogeny of the organs. The thymus begins to regress after puberty (Bloom et al., 1962), and  $F_1$  males would be expected to have larger absolute thymus weights. Seminal vesicles, as accessory sex organs, begin to develop at puberty (Turner, 1966). Although cytological evidence of spermatogenesis indicated that all  $DF_1$  males were sexually mature, seminal vesicles of some had not reached maximal development when sacrificed. Thus, very different interpretations of organ weight responses can be made depending on the measurement arbitrarily selected.

The regressions of organ weight against body weight (Figs. 4-7) showed that the consistent shifts in differences of mean organ weights were more a function of growth rate than of changes in organ weights. This is illustrated by a comparison of regressions of both absolute and relative organ weights to body weights.

A marked similarity appears in the regressions of absolute weights of both adrenals and testes on body weight (Figs. 4-5). Both show a positive slope. The fact that there is no significant difference among the slopes suggests that the groups were not different from each other. Experimental conditions apparently did not significantly affect the relationship of absolute organ weight to body weight. These figures substantiate the expectation that larger animals tend to have larger organs. Thus, we can expect the organs of the younger adults ( $DF_1$ ) to be smaller than organs of older adults (DP). This is shown by the left side of Fig. 9. If the stress syndrome had been acting in the dense population we would have expected the slope of adrenal weights to rise more steeply than that of the testes (Figs. 4 and 5).

A marked similarity of slopes also appears in the regressions of relative weights of adrenals and testes on body weight (Figs. 6-7). Both slopes are negative. There is a significant difference among the slopes of relative weights of adrenals ( $P, .04$ ) and of testes

( $P < .001$ ). The differences are accounted for by the slopes of the  $DF_1$  group, and suggest that our experimental conditions significantly affected the relationship of relative organ weight to body weight in the  $DF_1$  generation. But the similarity of the correlations

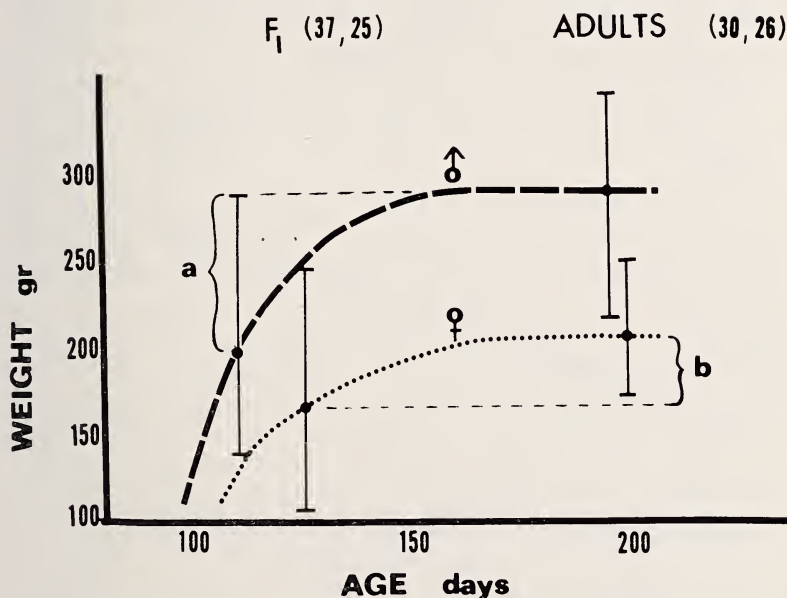


Fig. 10. Schematic diagram of growth rates in D cages based on bi-weekly weight averages. The vertical bars show the weight ranges within each group. The differences in mean weights between  $DF_1$  and D parents were (a) males, 82 g and (b) females, 39 g. N is given in parentheses for males and females respectively.

in Fig. 6-7 suggests that both relationships were affected by a common factor.

The factor that most affected relative weights was the denominator of the ratio (organ wt: body wt.) and not the numerator. Fig. 10 shows that  $DF_1$  males were a heterogeneous group with regard to growth characteristics. Animals at the lower end of the  $F_1$  weight range were younger, rapidly growing individuals. Ratios of relative organ weights calculated during this period tend to be high (dependent on the ontogeny of the organ), just as LWR's tend to be high because younger individuals are proportionally thinner. This is shown on the right side of Fig. 9. Animals at the higher end

of the  $F_1$  weight range were older individuals, whose growth rate had begun to level off. Both relative organ ratios and LWR's tend to decrease as individuals become older and proportionately heavier. The marked negative slopes of  $DF_1$  males in Figs. 6-7 reflect the heterogeneity of the sample and are primarily a function of differential growth characteristics within the  $F_1$  group. The regressions in Fig. 6-7 are linear representations of the inverse image of the growth curve shown in Fig. 10. The marked negative slope of  $DF_1$  males reflects the rapid growth phase shown in Fig. 10, and the moderate slopes of older adults reflect the phase of slower growth.

The differences in organ weights between  $DF_1$  and DP females were not as marked nor as consistent as for males (Tables 3-8). Absolute weights were not always smaller and relative weights were not always larger. This can be attributed to differential growth rates between the sexes. Males grew faster for a longer period of time than did the females (Fig. 10). The greater number of days required by females in all experimental conditions to grow from 100 to 180 g (Fig. 3) indicates that female growth rates decreased within this weight range. Biweekly weight records for females verify this fact. Fig. 10 shows that, when sacrificed, the weights of  $F_1$  females were more closely approaching maximum levels than were the weights of  $F_1$  males. The difference in maximum body weights between  $DF_1$  and DP males was 58 g, while the difference between maximum female body weights was only 5 g. Females of the  $DF_1$  and DP generations were more homogeneous in growth characteristics; therefore, the disparity in organ weights caused by differential growth rate was minimized.

A comparison of mean weights of organs is valid only when homogeneous samples are compared. In studies that involve field populations of unknown age, the homogeneity of samples is usually based on sexual maturity. However, sexual maturation precedes physical maturation (maximum somatic growth). For instance, Calhoun (1962) found that wild female Norway rats may begin to ovulate as early as 40 days of age even though reproductive behavior is not mature until between 80 to 115 days. He also showed that maximum growth (and minimum LWRs) did not occur until the age of 250 days. His study indicated that sexual maturation may occur more than 150 days prior to physical maturation in the Norway rat. The interval between sexual and physical maturation



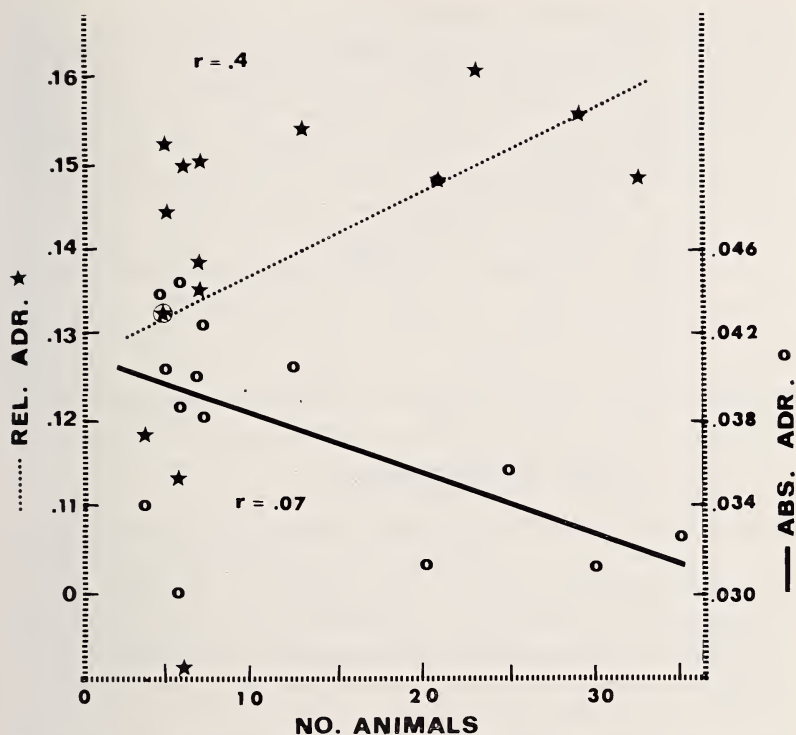


Fig. 11. Regressions of male relative and absolute adrenal weights correlated with the final number of rats per cage. Star within circle, 2 points.

varies widely between species, but the results of this study suggest that sexual maturity does not necessarily define comparable homogeneous samples. The consistent disparity in organ weights (Fig. 9) indicates that a comparison between  $DF_1$  and DP groups is not valid even though all animals were sexually mature.

The age structure of a population (ratio of young adults to older adults) therefore assumes great importance in the interpretation of responses of organ weights, as illustrated by the following results.

If the animals in this study had been trapped in a field population, and if the criterion for homogeneous samples was sexual maturity,  $F_1$  and parent animals would have been considered as a single group. When absolute weights of adrenals from  $DF_1$  and DP are pooled and the mean weights of all cages of all populations are plotted against the final number of animals in each cage, the

slope of the regression is negative (Fig. 11). The absolute weights of adrenals of  $DF_1$  rats were lower than their parents and there was a sufficient number of  $DF_1$  males to weight cage means in the direction evidenced by the  $DF_1$  group. However, the correlation coefficient ( $r$ , .07) indicates a random relationship between absolute weights of adrenals and density, despite the negative slope of the regression. On the other hand, when relative weights of adrenals from both generations were pooled and plotted in the same way, relative weights of adrenals correlated positively and significantly with population density ( $r$ , .45;  $P < .1$ ). The positive correlation shown in Fig. 11 could be logically interpreted as adrenal hypertrophy in response to density. It is, however, a spurious result of the age structure of the D population. Relative weights of adrenals of  $DF_1$  rats were higher than those of their parents because of differential growth characteristics, and because D cage means were numerically weighted in the direction of  $F_1$  males. No such correlation occurred when mean relative weights of organs of homogeneous groups (parent generation from each cage) were correlated with population density. The reduced rate of growth of the D populations, particularly of  $DF_1$  animals (Fig. 3), undoubtedly contributed to the greater strength of the positive correlation shown in Fig. 11.

The strength of the correlations shown in Fig. 11 is not as important as is the fact that the same adrenal glands from the same animals were used to demonstrate both a positive and a negative slope, as well as a significant and a non-significant relationship to population density. Inspection of mean organ weights (Table 3) and a comparison of the differences in weights between generations (Fig. 9) shows that similar contradictory slopes would result for most organs studied. Neither slope in Fig. 11 defines specific organ weight responses to population density, however. The contradictory slopes are a function of the age structure of this population.

Changing age structure during fluctuations of populations is well illustrated in the literature (Odum, 1959; Crowcroft, 1966). Newson (1963) found that a population of voles contained animals of all sizes only at the height of the breeding season. He also found that the variance of body weights, which is a measure of the heterogeneity of the sample, was high during the breeding season. The variance tended to fall during the non-breeding season as the age structure of the population became more homogeneous.

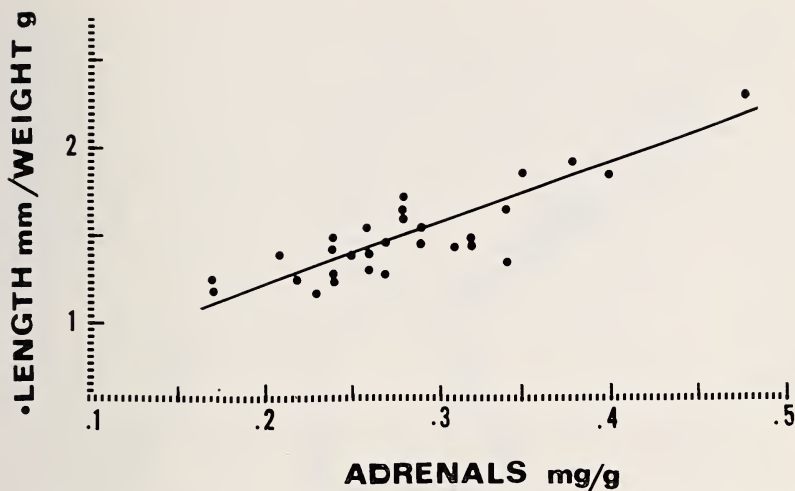


Fig. 12. Correlation of LWR and relative weight of adrenals of a field population of the cotton rat. ( $r$ , .97;  $P < .001$ ).

The following example illustrates how age structure and growth rate might result in a relationship that could be interpreted as “causal” rather than “coincidental”. During a study of brown lemmings in Alaska, samples that were collected in June in a declining population consisted of old, adult animals (Pitelka, 1957). Environmental conditions were described as favorable. Males and females evidenced a 35 and 30 per cent increase in weight, respectively, over a two-week interval. From mean weights and lengths that were presented, we calculated mean LWRs. They decreased from 2.9 to 2.2 for females and from 2.6 to 2.0 for males. Although Pitelka gave no organ weight data, relative organ weights would probably decrease as a function of the marked weight gain, just as LWR's decreased. A decrease in relative organ weights under these circumstances would be only coincidental to a decrease in population density.

The relationship between relative weights of organs and LWRs exists by virtue of their common denominator, body weight. Generally, relative organ weights will tend to decrease as LWR's decrease. Fig. 12 shows a predictably high positive correlation ( $r$ , .97;  $P < .001$ ) between relative weights of adrenals and LWRs in a field population of *Sigmodon hispidus*. Fig. 13 illustrates the similarity in the relationship of the same relative adrenal weights and LWRs

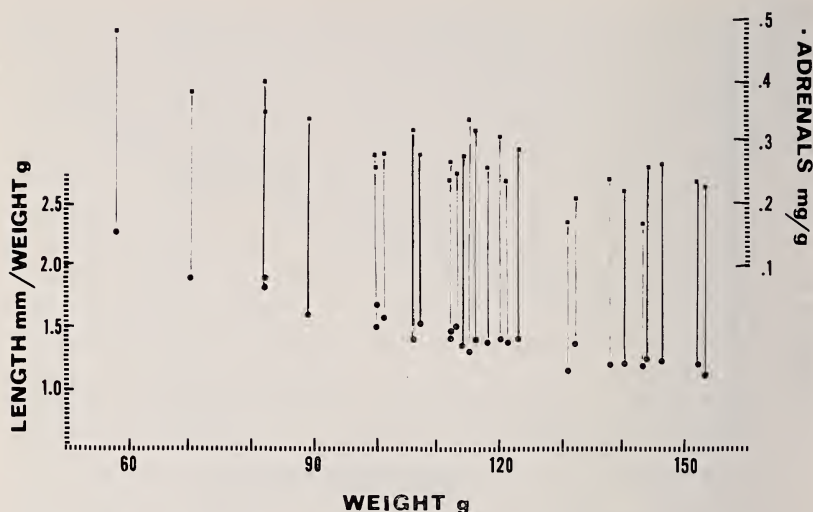


Fig. 13. Relative adrenal weights (squares) and length-to-weight ratios (circles), plotted against body weight for a field population of *Sigmodon hispidus*. Vertical lines connect points representing individual animals.

to individual body weights and shows that both relative organ ratios and LWRs tend to decrease as body weight increases. If high LWRs correspond with high relative organ weights, it is likely that growth rate is the primary factor involved.

The practicability of LWRs in defining a homogeneous group within a field population is also illustrated in Fig. 13. Age was necessarily an unknown factor; therefore LWRs were plotted against body weights. The curve levels off at approximately 100 g or at an LWR of approximately 1.5 or less. Animals above this weight and below this LWR constitute a homogeneous group. The point at which such a curve levels off may vary greatly, even for the same species, depending on environmental conditions. However, the LWR can be used to identify groups of animals of comparable somatic maturity. Once this is established, there is no need to convert absolute organ weights to relative weights in order to compensate for size differences. The conversion of organ weights of homogeneous groups may even lead to spurious conclusions if environmental conditions are different for the two samples being compared. A significant difference in LWRs indicates that a significant difference of relative organ weights in the same direction is meaningless.

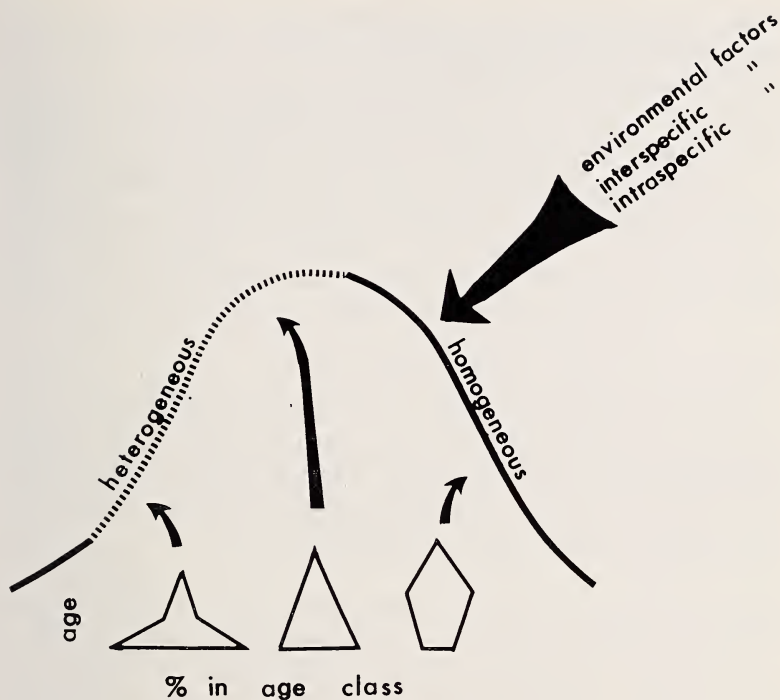


Fig. 14. Relationship of age distribution and any point in an expanding or declining population. Proportionally more sexually mature but somatically immature individuals are found in the increasing population.

Fig. 13 shows that the results of a comparison of adrenal weights may be misleading when the age structure of the population numerically weights a sample at either end of the maturity range. A schematic diagram of a population fluctuation and the changing age structure is presented in Fig. 14. When a sample consists primarily of young adults, a situation which might be encountered in an increasing population, mean relative weights of adrenals and LWRs tend to be higher and mean absolute weights of adrenals tend to be lower. On the other hand, relative adrenal weights and LWRs tend to be lower and absolute adrenal weights tend to be higher in a sample that consists primarily of older adults such as might be encountered in a decreasing population. Significant differences between mean weights of organs sampled at the peak and trough of a fluctuation may be more indicative of the age structure



of the two populations than of physiological responses of specific organs to environmental conditions.

Our conclusion is that in a study of natural populations great care must be taken to compare organ weights of similar animals. When organs of animals from populations of high and low density are compared, a homogeneous sample from each population must be used. The LWR is a parameter to ascertain this homogeneity. Without such a standard, relative and absolute organ weights may reflect the body weight and the age structure of the population instead of the density of the population.

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